

2 AF

PTO/SB/21 (07-06)

Approved for use through 09/30/2006. OMB 0651-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM (to be used for all correspondence after initial filing)	Application Number	09/646,835
	Filing Date	1/11/2001
	First Named Inventor	Multhoff et al.
	Art Unit	1643
	Examiner Name	Yaen
Total Number of Pages in This Submission	Attorney Docket Number	KNAUTHE-08713

ENCLOSURES (Check all that apply)		
<input checked="" type="checkbox"/> Fee Transmittal Form	<input type="checkbox"/> Drawing(s)	<input type="checkbox"/> After Allowance Communication to TC
<input checked="" type="checkbox"/> Fee Attached	<input type="checkbox"/> Licensing-related Papers	<input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences
<input type="checkbox"/> Amendment/Reply	<input type="checkbox"/> Petition	<input checked="" type="checkbox"/> Appeal Communication to TC (Appeal Notice, Brief, Reply Brief)
<input type="checkbox"/> After Final	<input type="checkbox"/> Petition to Convert to a Provisional Application	<input type="checkbox"/> Proprietary Information
<input type="checkbox"/> Affidavits/declaration(s)	<input type="checkbox"/> Power of Attorney, Revocation	<input type="checkbox"/> Status Letter
<input type="checkbox"/> Extension of Time Request	<input type="checkbox"/> Change of Correspondence Address	<input type="checkbox"/> Other Enclosure(s) (please identify below):
<input type="checkbox"/> Express Abandonment Request	<input type="checkbox"/> Terminal Disclaimer	
<input type="checkbox"/> Information Disclosure Statement	<input type="checkbox"/> Request for Refund	
<input type="checkbox"/> Certified Copy of Priority Document(s)	<input type="checkbox"/> CD, Number of CD(s) _____	
<input type="checkbox"/> Reply to Missing Parts/Incomplete Application	<input type="checkbox"/> Landscape Table on CD	
<input type="checkbox"/> Reply to Missing Parts under 37 CFR 1.52 or 1.53	Remarks Submitted herewith, in triplicate, is Appellant's Brief in furtherance of the Notice of Appeal filed June 5, 2006 and received in the U.S. Patent Office on June 7, 2006. Applicants also submit the required fee under Section 1.17(h). Please charge any additional fees, including any fees necessary for extensions of time, or credit overpayment to Deposit Account No. 08-1290.	

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT			
Firm Name	MEDLEN & CARROLL LLP		
Signature			
Printed name	J. Mitchell Jones		
Date	August 7, 2006	Reg. No.	44,174

CERTIFICATE OF TRANSMISSION/MAILING			
I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:			
Signature			
Typed or printed name	Mary Ellen Waite	Date	August 7, 2006

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

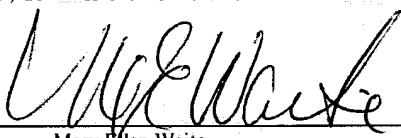
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Gabrielle Multhoff *et al.*
Serial No.: 09/646,835
Filed: 01/11/01
Entitled: **Use of Hsp70 Proteins**

Group No.: 1643
Examiner: Christopher Yaen

APPELLANTS' BRIEF
APPEAL NO.:

Mail Stop Appeal Brief - Patents
Commissioner for Patents and Trademarks
P.O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)(1)(i)(A)	
I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop Appeal Brief - Patents , Commissioner for Patents and Trademarks P.O. Box 1450 Alexandria, VA 22313-1450	
Dated: <u>August 7, 2006</u>	By:  Mary Ellen Waite

Sir or Madam:

This Brief is in furtherance of the Notice of Appeal filed June 5, 2006.

The fees required under § 1.17(h) and any required Petition for Extension of Time for filing this Brief and fees therefore are dealt with in the accompanying
TRANSMITTAL OF APPEAL BRIEF.

This Brief is transmitted in triplicate. [37 C.F.R. § 41.37(c)].

08/10/2006 YPOLITE1 00000001 09646835

01 FC:2402

250.00 OP

This Brief contains these items under the following headings and in the order set forth below [37 C.F.R. § 41.37(c)]:

I. REAL PARTY IN INTEREST.....3

II. RELATED APPEALS AND INTERFERENCES.....3

III. STATUS OF CLAIMS..... 3

IV. STATUS OF AMENDMENTS..... 4

V. SUMMARY OF CLAIMED SUBJECT MATTER.....4

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL.....5

VII. ARGUMENT.....5

VIII. CLAIMS APPENDIX.....19

IX. EVIDENCE APPENDIX.....23

X. RELATED PROCEEDINGS APPENDIX.....24

XI. CONCLUSION.....25

I. REAL PARTY IN INTEREST

The real party in interest is Dr. Gabriele Multhoff.

II. RELATED APPEALS AND INTERFERENCES

There are no known related appeals or interferences known to Appellants, Appellants' legal representative, or the Assignee.

III. STATUS OF CLAIMS

Claims 1 - 30 were filed in the original application, a National Phase Entry from PCT Application EP99/02165. During prosecution of the application, Claims 31 –87 were added, Claims 1-48, 50-60, and 78-82 cancelled, and claim 49 withdrawn. Claims 61-77 and 83-87 are currently pending and have been rejected by the Office in the Final Office Action dated April 4, 2006. Therefore, Claims 61-77 and 83-87 are pending in this appeal. No other claims are pending. Thus, Appellants appeal the Final Office Action of April 4, 2006. The Claims, as they now stand, are set forth in the Claims Appendix.

IV. STATUS OF AMENDMENTS

Appellants' Response to the Office Action mailed March 1, 2006 has been entered per the Final Office Action dated April 4, 2006. Amendments to the claims that were made in the March 1, 2006 Response were acknowledged in the Final Office Action dated April 4, 2006. Thus, there are no pending amendments not entered into the record.

V. SUMMARY OF CLAIMED SUBJECT MATTER

This invention relates to the use of Hsp70 polypeptides to activate Natural Killer Cells (NK-cells) ex vivo. See, English Translation of PCT Application at 1. In particular, the invention relates to the use of a protein, protein fragment, or polypeptide selected from the group consisting of a Hsp70 protein of SEQ ID NO.: 1, a C-terminal fragment of Hsp70, wherein said fragment comprises amino acids 384-641 of SEQ ID NO.: 1, and a polypeptide having 70% or greater homology to amino acids 384-641 of SEQ ID NO.: 1. Id. at 2-3. Preferably, the protein, fragment, or polypeptide induce an immune response by the treated NK cells. Id. at 5. Preferably, the immune response increases cytolytic activity of the NK cells or stimulates proliferation of the NK cells. Id. The Hsp70 polypeptides are added to a physiological suspension containing NK cells, such as a peripheral mononuclear blood cell fraction. Id. at 14, Example 1. The cell suspension can contain diseased cells, including leukemia cells, lymphoma cells, tumor cells, metastasizing cells of solid tumors and cells from a virally, mycotically and/or bacterially infected patient. Id. at 5, 15, Example 2. The NK-cells are thereby induced to attack Hsp70 expressing diseased cells, such as tumor cells. Id. at 15-18, examples 2 and 3.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

There is one ground of rejection to be reviewed on appeal:

Issue 1 – Whether Claims 61-77 and 83-87 are supported by an adequate written description.

VII. ARGUMENT

Issue 1 - Claims 61-77 and 83-87 are supported by an adequate written description.

Claims 61-77 and 83-87 are rejected under 35 U.S.C. 112, first paragraph, as allegedly lacking an adequate written description. As explained below, the Examiner's rejection is unsupportable because 1) Eli Lilly and related cases are not controlling and 2) the Examiner has ignored the Written Description Guidelines promulgated by the Office.

1. Eli Lilly is not controlling

The Examiner's rejection relies heavily on Eli Lilly and related cases. Final Office Action dated April 4, 2006 at p. 3-4. The Examiner states that "the Federal Circuit held that a generic statement that defines a genus of nucleic acids by only their functional activity does not provide an adequate written description of the genus." *Id.* at 3. The Examiner goes on to state "by analogy, a generic statement that defines a genus of polypeptides having 70% or greater homology to amino acids 384-641 of SEQ ID NO.:1 by only their common ability to induce an immune response by NK cells, wherein the response increases cytolytic activity of the NK cells or stimulates NK cell proliferation does not adequately describe the genus as a whole."

As a preliminary matter, Eli Lilly is not analogous as suggested by the Examiner. Neither the specification nor the claims of the patent addressed by the Federal Circuit in Eli Lilly contained the sequence for human insulin. Regents of the University of California v. Eli Lilly, 43 USPQ2d 1398 (Fed. Cir. 1997). In the instant, the sequence of a Hsp70 is provided in the both the specification and the claims and, as described in more detail below, was well known in the art as of the priority date of the application. Thus, applicants have not attempted to define a genus of nucleic acids by only their functional activity. Instead, applicants have disclosed and referred to specific sequences. Thus, the analogy the Examiner attempts to draw has no merit.

The Examiner next cites Noelle v. Lederman for the proposition that "the Federal Circuit has decided that a patentee of a biotechnological invention cannot necessarily claim a genus after only describing a limited number of species because there may be unpredictability in the results obtained from other species other than those specifically enumerated." Final Office Action at 4. However, Noelle dealt with an application in which the claims were directed to monoclonal antibodies and in which a only a single monoclonal antibody epitope was disclosed. Noelle v.

Lederman, 69 USPQ2d 1508 (Fed. Cir. 2004). Noelle did not address the situation where specific sequences are provided in the application and claims and known in the art. Thus, Noelle is not applicable to the instant case either.

The Examiner does admit that “Applicants have pointed to several species of HSPs that may fall within the genus of at least 70% homologous to amino acids 384-641 of SEQ ID NO:1.” Final Office Action at 4. The Examiner also does not dispute, and simply dismisses the evidence provided by Applicants, that Milner and Campbell and Multhoff et al. teach that sequences within the claimed genus were known in the art. *Id.* at 3. In fact, the Examiner appears to accept that Milner and Campbell “teaches Hsp70-Hom and Hsp70-B both of which are at least 70% homologous to amino acids 384-641” and that Multhoff et al. “provides an overview of the Hsp70 multigene family.” *Id.*

For the convenience of the Office, a copy of the Milner and Campbell reference is provided herewith. As demonstrated in Example 1 and shown in Fig. 1A of the present application, the proliferation and thus activity of NK cells could be stimulated by recombinant human Hsp70 as well as by the C-terminal fragment of homologous protein rHsp70homC (see, also, the Specification at page 16, line 2 through the completion of the second full paragraph).

Thus, the Applicants have actually reduced to practice a sequence that is 70% or greater in homology to amino acids 384-641 of SEQ ID NO: 1. Furthermore, the enclosed Milner and Campbell reference provides evidence that already in 1990, i.e. eight years prior to the effective filing date of the present application, several members of the Hsp70 family were known, which can be used in accordance with the teaching of the present invention.

For example, besides the Hsp70-Hom protein the Milner and Campbell reference discloses in Fig. 4 another homologous Hsp70 protein, i.e. Hsp70-B', the amino acid sequence of which has a homology of about 74% to amino acids 384-641 of the reference Hsp70 protein. Furthermore, reference is made to the publications by the inventor Multhoff et al., *Cell Stress & Chaperones* 1 (1996), 6-11 and Multhoff et al., *Biol. Chem.* 379 (1998), 295-300, both referenced at Page 1 of the present application, which provide an overview concerning Hsp70 multigene family including various citations. Thus, besides the fact that applicant actually reduced to practice homologous sequences of amino acids 384-641 of SEQ ID NO: 1 the prior art at the time the application was filed provided already a vast source of the genus of Hsp70 sequences that are encompassed in the claimed method.

In this respect, the Examiner's attention is respectfully directed to the Federal Circuit's recent holding in Falkner v. Inglis, 448 F.3d 1357; 79 U.S.P.Q.2D (BNA) 1001 (Fed. Cir. 2006). In that case, the Federal Circuit specifically held that "Eli Lilly does not set forth a per se rule that whenever a claim limitation is directed to a macromolecular sequence, the specification must always recite the gene or sequence, regardless of whether it is known in the prior art." *Id.* at 1367. The Federal Circuit went on to explain that:

Thus, "[w]hen the prior art includes the nucleotide information, precedent does not set a per se rule that the information must be determined afresh." *Id.* at 1358. Rather, we explained that:

The descriptive text needed to meet these requirements varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence. The law must be applied to each invention that enters the patent process, for each patented advance is novel in relation to the state of the science. Since the law is applied to each invention in view of the state of relevant knowledge, its application will vary with differences in the state of knowledge in the field and differences in the predictability of the science.
Id. at 1357.

Indeed, a requirement that patentees recite known DNA structures, if one existed, would serve no goal of the written description requirement. It would neither enforce the quid pro

quo between the patentee and the public by forcing the disclosure of new information, nor would it be necessary to demonstrate to a person of ordinary skill in the art that the patentee was in possession of the claimed invention. As we stated in *Capon*, "[t]he 'written description' requirement states that the patentee must describe the invention; it does not state that every invention must be described in the same way. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution." *Id.* at 1358. Indeed, the forced recitation of known sequences in patent disclosures would only add unnecessary bulk to the specification.

Id. at 1367-68. The Federal Circuit then specifically held that "where, as in this case, accessible literature sources clearly provided, as of the relevant date, genes and their nucleotide sequences (here "essential genes"), satisfaction of the written description requirement does not require either the recitation or incorporation by reference (where permitted) of such genes and sequences." *Id.* In the instant case, sequences within the claims were known in the art and reference sequences were described in the specification and identified in the claims. That is all the written description that is needed.

Additionally, Applicants note that the priority date for the present application is March 27, 1998. The priority date for the patent addressed by the Federal Circuit in Eli Lilly was September 12, 1979 (i.e., U.S. Pat. No. 4,431,740). Regents of the Univ. of California v. Eli Lilly, 119 F.3d 1559, 1562 (Fed. Cir. 1997). As held by the Federal Circuit in *Falkner*, "Since the law is applied to each invention in view of the state of relevant knowledge, its application will vary with differences in the state of knowledge in the field and differences in the predictability of the science." Falkner, 448 F.3d at 1367-68. Surely, the state of art molecular biology had advanced considerably in the approximately 20 years elapsed between the filing the patent at issue in Eli Lilly and the present application. The simple fact is that the macromolecules utilized in the instant claims were both known when the application was filed and described in detail in the application itself. These sequences are representative of a broader genus, which any person of skill in the art can identify. Indeed, even if a person skilled in the art did not want to rely on the available Hsp70 protein species available at the time the present application was filed, the specification provides sufficient guidance how to produce and test appropriate derivatives of the exemplified Hsp70 protein and fragments thereof (see, e.g., the Specification at Page 3, second to fourth full paragraphs, Page 4, the paragraph bridging to Page 5 and Example 1 which describes spanning experiments for testing the capability of Hsp70 species to increase the proliferation of NK cells).

2. Application of the Written Description Guidelines compels allowance of the claims

Applicants respectfully refer the Examiner to the USPTO's "Synopsis of Application of Written Description Guidelines", and in particular to Example 14, pages 53-55. Consideration of the Examples in the Written Description Guidelines establishes that the claims are supported by an adequate written description.

The claim of Example 14 of the Written Description Guidelines recites a protein having SEQ ID NO:3 and variants thereof that are at least 95% identical to SEQ ID NO:3 and catalyze the reaction of A->B. The disclosure of Example 14 provides a single species (SEQ ID NO:3) that has actually been reduced to practice, and describes an assay for identifying the variants having the desired catalytic activity. The analysis considers (1) whether the members of genus vary substantially from each other; and (2) whether the disclosed species is representative of the members of the genus; in order to determine whether one of skill in the art would determine if the applicant was in possession of the necessary common attributes possessed by the members of the genus.

For Example 14, it was determined that the member species did not substantially vary since the variants have 95% identity or greater to the reference sequence, and also possess the catalytic activity. It was also determined that the disclosed species was representative since all members must have at least 95% structural identity to SEQ ID NO:3. The analysis determined that one of skill in the art would conclude that the applicant was in possession of the necessary common attributes possessed by the members of the genus, and therefore the disclosure in this Example meets the written description requirement.

Applicants submit that the instant claims can be analyzed in a similar manner to that provided in Example 14. First, the polypeptides do not substantially vary as members of a genus since they all have at least 70% homology to SEQ ID NO:1 and must possess the activity of inducing an immune response by NK cells. Second, the polypeptide having SEQ ID NO:1 is a representative species of the genus since all polypeptides must have at least 70% homology to this sequence. Therefore, one of skill in the art would conclude that the Applicants were in possession of the necessary common attributes possessed by the members of the genus, and therefore the instant specification meets the written description requirement for these claims.

The Examiner seems to attempt to draw a distinction between the 70% limitation in the present claims and the 95% homology limitation in Example 14. However, this distinction is without meaning. The Written Description Guidelines focus on the provision of a reference sequence and a limitation as to function. Both of these limitations are present in the instant claims. As a result, the Written Description Guidelines are persuasive authority that the instant claims are allowable.

VIII. CLAIMS APPENDIX

1-48. (Cancelled)

49. (Withdrawn) The method of claim 43, wherein said infectious diseases are viral, mycotic or bacterial diseases.

50-60. (Cancelled)

61. (Previously presented) A method for the ex vivo activation of NK- cells, comprising: contacting NK cells in physiological suspension with an isolated and uncomplexed protein, protein fragment, or polypeptide selected from the group consisting of a Hsp70 protein of SEQ ID NO.: 1, a C-terminal fragment of Hsp70, wherein said fragment comprises amino acids 384-641 of SEQ ID NO.: 1, and a polypeptide having 70% or greater homology to amino acids 384-641 of SEQ ID NO.: 1, wherein said isolated protein, fragment, or polypeptide induce an immune response by NK cells, and further said response increases cytolytic activity of the NK cells or stimulates proliferation of the NK cells.

62. (Previously presented) The method of claim 61, wherein said activation of said cells further comprises stimulation of proliferation and/or an increase in cytotoxicity.

63. (Previously presented) The method of claim 61, wherein said physiological suspension containing NK cells comprises a peripheral mononuclear blood cell fraction or fractions thereof.

64. (Previously presented) The method of claim 61, wherein said suspension further comprises cells expressing cell-surface Hsp70.

65. (Previously presented) The method of claim 64, wherein said expressing cells comprise diseased cells from a patient.

66. (Previously presented) The method of claim 65, wherein said diseased cells are selected from the group consisting of leukemia cells, lymphoma cells, tumor cells, metastasizing cells of solid tumors and cells from a virally, mycotically and/or bacterially infected patient.

67. (Previously presented) The method of any one of Claims 61-66, wherein said contacting is carried out for at least 3 hours.

68. (Previously presented) The method of claim 67, wherein said contacting is carried out for 4 days.

69. (currently amended) The method of claim 67, wherein ~~said conditions~~ said contacting further comprise addition of cytokine.

70. (Previously presented) The method of claim 69, wherein the cytokine is an interleukin.

71. (Previously presented) The method of claim 70, wherein said interleukin is selected from the group consisting of IL-2, IL-12 and IL-15.

72. (Previously presented) A method for the in vivo activation of the immune system in a patient in need thereof comprising:

- i) administering to said patient a pharmaceutically effective amount of NK cells obtained by the method of claim 61; and
- ii) optionally administering to said patient, concurrently or subsequently, a pharmaceutically effective amount of an isolated and uncomplexed protein, protein fragment, or polypeptide selected from the group consisting of a Hsp70 protein of SEQ ID NO: 1, a C-terminal fragment of Hsp70, wherein said fragment comprises amino acids 384-641 of SEQ ID NO.: 1, and a polypeptide having 70% or greater homology to amino acids 384-641 of SEQ ID NO.: 1, wherein said isolated protein, fragment, or

polypeptide induces an immune response by NK cells, and wherein said response increases cytolytic activity of the NK cells or stimulates proliferation of the NK cells.

73. (Previously presented) The method of claim 72, where said patient is suffering from a disease selected from the group consisting of cancerous, infectious and autoimmune disease.

74. (Previously presented) The method of claim 72, further comprising administering a cytokine.

75. (Previously presented) The method of claim 74, wherein said cytokine is an interleukin.

76. (Previously presented) The method of claim 75, wherein said interleukin is selected from the group consisting of IL-2, IL-12 and IL-15.

77. (Previously presented) The method of claim 73, wherein said cancerous disease is selected from the group consisting of tumors, solid tumors, metastatic tumors, leukemias and lymphomas.

78-82. (Canceled).

83. (Previously presented) A method for in vivo activation of the immune system in a patient in need thereof comprising administering to said patient a pharmaceutically effective amount of an isolated and uncomplexed protein, protein fragment, or polypeptide selected from the group consisting of a Hsp70 protein of SEQ ID NO.:1, a C-terminal fragment of Hsp70, wherein said fragment comprises amino acids 384-641 of SEQ ID NO.: 1, and a polypeptide having 70% or greater homology to amino acids 384-641 of SEQ ID NO.: 1; wherein said isolated protein, fragment, or polypeptide induces an immune response by NK cells, and wherein

said response increases cytolytic activity of the NK cells or stimulates proliferation of the NK cells.

84. (Previously presented) The method of claim 83, where said patient is suffering from a disease selected from the group consisting of cancerous, infectious and autoimmune disease.

85. (Previously presented) The method of claim 83, further comprising administering a cytokine.

86. (Previously presented) The method of claim 85, wherein said cytokine is an interleukin.

87. (Previously presented) The method of claim 86, wherein said interleukin is selected from the group consisting of IL-2, IL-12 and IL-15.

IX. EVIDENCE APPENDIX

Per 37 C.F.R. §41.37(c)(ix), a copy of Milner and Campbell, Structure and expression of the three MHC-linked HSP70 genes, Immunogenetics 32:242-251 (1990) is provided with the present appeal brief.

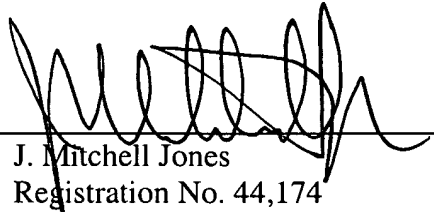
X. RELATED PROCEEDINGS APPENDIX

There are no related proceedings.

XI. CONCLUSION

For the foregoing reasons, it is submitted that the Office's rejection of Claims 61-77 and 83-87 is erroneous, and reversal of the rejection is respectfully requested. Appellant requests either that the Board render a decision as to the allowability of the claims, or alternatively, that the application be remanded for reconsideration by the Office.

Dated: August 7, 2006



J. Mitchell Jones
Registration No. 44,174

MEDLEN & CARROLL, LLP
101 Howard St., Ste. 350
San Francisco, CA 94105
415-904-6500

Structure and expression of the three MHC-linked *HSP70* genes

Caroline M. Milner and R. Duncan Campbell

MRC Immunogenetics Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, England

Received May 16, 1990; revised version received June 28, 1990

Abstract. A duplicated locus encoding the major heat shock-induced protein *HSP70* is located in the major histocompatibility complex (MHC) class III region 92 kilobases (kb) telomeric to the *C2* gene. Nucleotide sequence analysis of the two intronless genes, *HSP70-1* and *HSP70-2*, has shown that they encode an identical protein product of 641 amino acids. A third intronless gene, *HSP70-Hom*, has also been identified 4 kb telomeric to the *HSP70-1* gene. This encodes a more basic protein of 641 amino acids which has 90% sequence similarity with *HSP70-1*. In order to investigate the expression of the three (MHC)-linked *HSP70* genes individually by northern blot analysis, we have isolated locus-specific probes from the 3' untranslated regions of the genes. The *HSP70-1* and *HSP70-2* genes have been shown to be expressed at high levels as a ~2.4 kb mRNA in cells heat-shocked at 42 °C. *HSP70-1* is also expressed constitutively at very low levels. The *HSP70-Hom* gene, which has no heat shock consensus sequence in its 5' flanking sequence, is expressed as a ~3 kb mRNA at low levels both constitutively and following heat shock.

Introduction

Heat shock proteins, or stress proteins, are expressed in response to heat shock and a variety of other stress stimuli including oxidative free radicals and toxic metal ions (for reviews see Lindquist 1986; Lindquist and Craig 1988). This response has been observed in all species examined to date. The family of stress proteins of *M*_r 70000, *HSP70*, is probably the most predominant and is highly conserved throughout evolution. The amino acid sequence similarities between eukaryotic *HSP70*s range from

60%–78% and there is 47% similarity between human *HSP70* and the *Escherichia coli* homologue *dnaK* (Hunt and Morimoto 1985).

Two independent studies (Goate et al. 1987; Harrison et al. 1987) using somatic cell hybrid analysis and genomic Southern blotting to detect restriction fragment length polymorphisms have demonstrated the presence of multiple autosomal loci for *HSP70* in the human genome. These were shown to lie on chromosomes 6, 14, 21, and at least one other autosome. The chromosome 6 *HSP70* loci were localized to a region on the short arm close to the major histocompatibility complex (MHC). During characterization of the class III region of the MHC for additional loci (Sargent et al. 1989a, b) a duplicated locus encoding *HSP70* was found between the complement and tumor necrosis factor (*TNF*) genes (Fig. 1A). The *HSP70* loci are 12 kilobases (kb) apart and lie 92 kb telomeric of the *C2* gene. In addition, a region of similarity was localized to a segment of DNA lying ~4 kb telomeric to the first copy of the duplicated *HSP70* locus. *HSP70* loci have also been mapped to the equivalent region of the rat (Wurst et al. 1989) and caprine (Cameron et al. 1990) MHCs.

The human *HSP70* multigene family encodes several highly conserved proteins with structural and functional properties in common, but which vary in their inducibility in response to metabolic stress. The structure of two of these has been completely determined (Wu et al. 1985; Hunt and Morimoto 1985; Leung et al. 1990). The *HSP70* sequence determined by Hunt and Morimoto (1985) is encoded by one of the genes (*HSP70-1*) located in the MHC (Harrison et al. 1987; Sargent et al. 1989b). This sequence shares 77% similarity with a more basic *HSP70* protein, *HSP70B'* (Leung et al. 1990). Other members of the *HSP70* protein family include a clathrin uncoating ATPase of *M*_r 72000 (Chappell et al. 1986) and a glucose-regulated protein (GRP78) of *M*_r 78000 (Ting and Lee 1988). In addition to the stress-induced proteins, the human genome also contains at least one *HSP70* cognate

The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession numbers M34267-9.

Address correspondence and offprint requests to: R. D. Campbell.

BEST AVAILABLE COPY

gene (*HSC70*; Dworniczak and Mirault 1987) which is constitutively expressed and shares 82% similarity in its amino acid sequence with *HSP70*.

In order to define the structure of the three MHC-linked *HSP70* loci and to determine whether they were identical to previously identified members of the *HSP70* family, we determined their nucleotide sequences. In addition, we have isolated locus-specific probes from the 3' untranslated regions of the three genes in order to investigate their expression both constitutively and in response to heat shock.

Materials and methods

Cloning and nucleotide sequence analysis. A 5.6 kb *Eco* RI/*Hind* III fragment containing the *HSP70-1* gene and a 3.6 kb *Pvu* II fragment containing the *HSP70-2* gene were isolated from cos H92 and cos 181 (Sargent et al. 1989a), respectively (Fig. 1B). These fragments were cloned into *Pvu* II-cut pATX and designated pHR-5.6 and pP-3.6,

respectively. A 2.4 kb *Bam* HI/*Hind* III fragment containing the coding sequence and 3' untranslated region of *HSP70-1* and a 0.4 kb *Bam* HI/*Nco* I fragment containing the 5' flanking and 5' untranslated region of this gene were isolated from clone pHR-5.4. These were subcloned into *Bam* HI/*Hind* III and *Bam* HI/*Hinc* II-cut Bluescript KS⁺ vector (clones BS2.4-1 and BS0.4-1). Similarly, a 2.6 kb *Bam* HI/*Pvu* II fragment containing the coding and 3' untranslated sequence of *HSP70-2* and a 0.9 kb *Bam* HI/*Pvu* II fragment encompassing the 5' flanking and 5' untranslated sequence were isolated from pP-3.6 and cloned into *Bam* HI/*Hinc* II-cut Bluescript vector (clones BS2.6-2 and BS0.9-2). The homologous region was isolated from cos H92 in two overlapping fragments: a 4 kb *Bam* HI/*Nco* I fragment which was cloned into *Bam* HI/*Hinc* II-cut Bluescript SK⁺ (BS4.0-H), and a 1.3 kb *Bam* HI/*Hind* III fragment which was cloned into Bluescript KS⁺ (BS1.3-H; Fig. 1B). Some smaller fragments from the homologous region were further subcloned into *Sma* I-cut M13 mp10. Single-stranded DNA was recovered from the Bluescript or M13 subclones and sequenced by the dideoxy nucleotide chain termination method using Sequenase (US Biochemicals, Cleveland, Ohio). In the case of the Bluescript clones the helper phage M13K07 was used for ssDNA recovery, in the presence of kanamycin. Nucleotide sequence was obtained using the M13 universal primer or specific oligonucleotide primers, which in the case of the *HSP70-1* and *HSP70-2* genes were based on the sequence published by

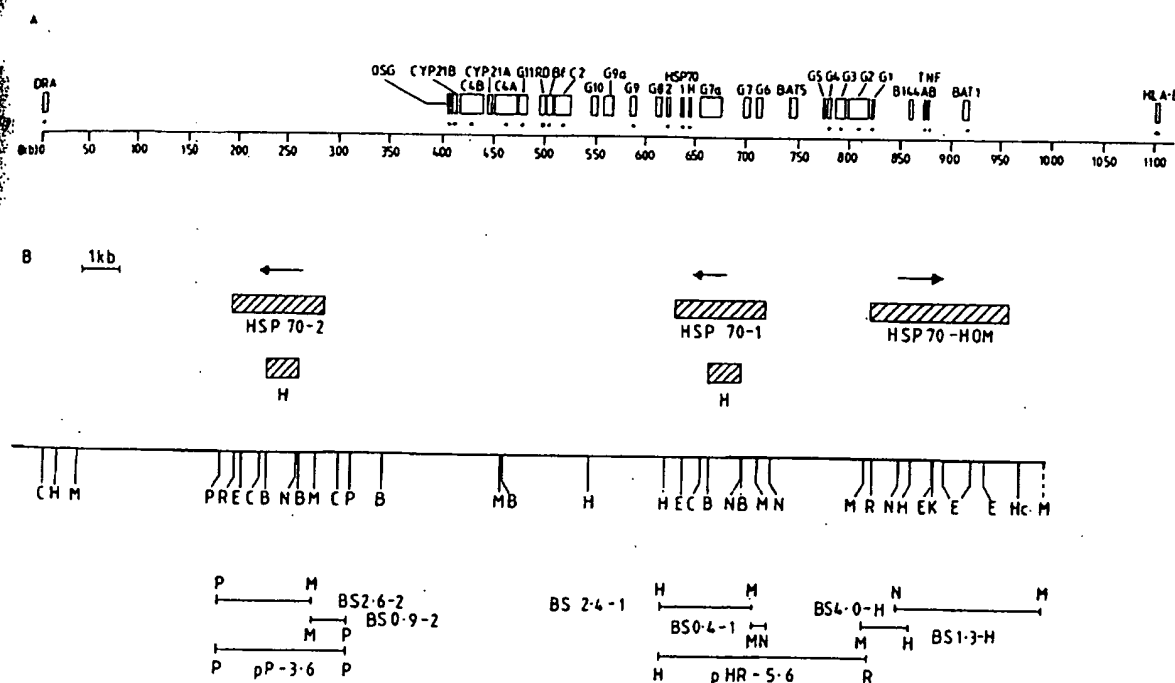


Fig. 1A. Molecular map of the MHC class III region, showing the position of the *HSP70* loci in relation to the other genes identified in this region (Sargent et al. 1989a). Open boxes at the top of the figures represent the locations of genes. The directions of transcription of the genes are shown by arrows, where these have been determined. The *HSP70* loci lie 92 kb telomeric to the *C2* gene. *HSP70-1* and *HSP70-2* are 11 kb apart, and the *HSP70-Hom* gene is 4 kb telomeric to *HSP70-1*. The genes *G2*, *G3*, *G5*, *G7a*, *G9*, *G9a*, and *G10* have also been designated *BAT2*, *BAT3*, *BAT4*, *BAT6*, *BAT7*, *BAT8*, and *BAT9*, respectively (Spies et al. 1989). Details of the *RD* gene and the *OSG* can be found in Levi-Strauss and co-workers (1988) and Morel and co-workers (1989). B Restriction map of that part of the MHC class III region containing the *HSP70* loci, which was isolated in overlapping cosmid clones 181 and H92. Restriction sites are shown for the enzymes *Bam* HI (M), *Bgl* II (B), *Cla* I (C), *Eco* 0109 (E), *Eco* RI (R), *Hinc* II (Hc), *Hind* III (H), *Nco* I (N), and *Pvu* II (P). The *Bam* HI site on the right of the map, shown by a dotted line, was formed by ligation of insert to vector in cos H92. The positions of the *HSP70* loci are shown by hatched boxes at the top of the figure, and the directions of transcription of the genes are indicated by arrows. The 0.9 kb *Bgl* II probe, H, is defined by hatched boxes. The bars at the bottom of the figure represent the regions cloned in pATX and Bluescript vectors.

BEST AVAILABLE COPY

HSP70-1 CGCCATGGAGACCAACACCCCTCCACCGCCACTCCCTCTCTCAGGGTCCCTGTCCCTCCAGTGAATCCAGAGACTCTGGAGA -184
HSP70-2 T A C

HSP70-1 GTTCTGAGCAGGGGGCGGCACTCTGGCTCTGATTCCTCAAGGAAGGCTGGGGGGCAGGACGGGAGCGGAAACCCCTGGATATTCCTG -194
HSP70-2 A C C

HSP70-1 ACCTGGCAGCCTCATCGAGCTCGGTGATTCCTCAGAAGGGAAGGCGGGTCTCCGTGACGACTTATAAAGCCGAGGGGCAAGCGGTC -4
HSP70-2 T G AC GC G

HSP70-1 CGGATAACGGCTAGCCTGAGGAGCTGCTGGGACAGTCCACTACCTTTTCGAGAGTGACTCCCGTTCTCCCAAGGCTTCCAGAGCGAAC -87
HSP70-2 A C GG G T G C C

HSP70-1 CTGTGCGGCTGCAGGACCGGGCGGCTCGAGTTTCGGCGTCCGGAAGGACCGAGCTCTTCTCGCGGATCCAGTGTTCGGTTTCAGCCCC -177
HSP70-2 T T T C T G C C G

HSP70-1 CAATCTCAGAGCGGAGCGGACAGAGGAGGGAACCGGATGGCCAAAGCGCGGATCGGATCGAGCTGGGACCACTACTCTCTGC -267
HSP70-2 G C C

HSP70-1 V G V F O H G K V E I I A N D O G N R T T P S Y V A F T D -265
HSP70-2 GTGGCGTGTTCACACCGCAAGGTGGAGATCATCGCAACGACGAGGCAACCGCACCACCCAGCTACGTGGCGTTTCAGGGACAC

HSP70-1 E R L I G D A A K N O V A L N P O N T V F D A K R L I G R K -447
HSP70-2 GAGCGGCTCATCGGGATCGCGCAAGAACAGGTGGCGTGAACCCGAGAACACCGTGTTCAGCGAAGCGCTGATTGGCGCAAG C

HSP70-1 F G D P V V O S D M K H W P F O V I N D G D K P K V O V S Y -537
HSP70-2 TTCGGGACCGGCTGGTGCAGTGGACATGAACGACGCGCTTTCAGGTGATCAACGACGGAGACAAGCCCAAGGTGACGTTGAGCTAC

HSP70-1 K G E T K A F Y P E E I S S M V L T K M K E I A E A Y L C Y -627
HSP70-2 AAGGGGAGACCAAGGCATTCTACCCGAGGAGATCTGTCATGGTGTGACCAAGATGAAGGAGATCGCGGAGGCGTACCTGGGCTAC

HSP70-1 P V T N A V I T V P A Y F N D S O R O A T K D A G V I A C L -717
HSP70-2 CCGGTGACCAACCGGCTGATCAGCGTCCGCGCTACTTCAACGACTCGCAGCGCCAGGCAAGGATCGGGTGTGATCGCGGGGCTC

HSP70-1 N V L R I I N E P T A A A I A Y G L D R T G K G E R N V L I -807
HSP70-2 AACGTGCTGCGGATCATCAACGAGCCACGCGCGCGGCTACCGCTACGGCTGGACAGAACGGGCAAGGGGAGCGCAACGTGCTCATC C G

HSP70-1 F D L G G G T F D V S I L T I D D G I F E V K A T A G D T H -897
HSP70-2 TTTGACCTGGCGGGGGACCTTCGAGTGTCCATCTGACGATCGAGCAGCGCATCTTCAGGTGAAGGCCACGGCGGGGACACCCAC

HSP70-1 L G G E D F D N R L V N H F V E E F K R K H K K D I S O N K -987
HSP70-2 CTGGTGGGAGGACTTTGACAACAGGCTGCTGAACCACTCTGTTGGAGGACTTCAAGAGAAAACACAAGAAGGACATAGCCAGCAACAAG

HSP70-1 R A V R R L R T A C E R A K R T L S S S T O A S L E I D S L -1077
HSP70-2 CGAGCGTGAGGCGGCTGCGCAGCGCTCGGAGAGGGCAAGAGGACCTGTCTGTCAGCAGCCAGGCGAGCTGGAGATCGACTCCTC

HSP70-1 F E C I D F Y T S I T R A R F E E L C S D L F R S T L E P V -1167
HSP70-2 TTTGAGGGCATCGACTTCTACAGTCCATCAGCGGCGAGGTTGAGGAGCTGTGCTCGACCTGTTCCGAAGCACCTGGAGCGCGTG

HSP70-1 E K A L R D A K L D K A O I H D L V L V G G S T R I P K V O -1257
HSP70-2 GAGAAGGCTCTGCGGAGCGCAAGCTGGACAAAGGCGGAGATTACGAGCTGCTGCTGGGGGCTCCACCGCATCCCAAGGTGCAG

HSP70-1 K L L O D F F N G R D L N K S I N P D E A V A Y G A A V O A -1347
HSP70-2 AAGCTGCTGAGGACTTCTCAACGGGCGGACCTGAACAAGAGCATCAACCCGACGAGGCTGTGGCTACGGGGCGGCGTGACGGG

HSP70-1 A I L M C D K S E N V O D L L L L D V A P L S L G L E T A C -1437
HSP70-2 GCCATCCTGATGGGGACAAGTCCGAGAAGTGCAGGACCTGCTGCTGAGAGTGGCTCCCTGTCGCTGGGGCTGGAGACGGCGGA

HSP70-1 G V M T A L I K R N S T I P T K O T O I F T T Y S D N O P G -1527
HSP70-2 GGCGTGATGACTGCCCTGATCAAGCGCAACTCCACCATCCCCACAGGAGAGCGAGATCTTACCACTACTCCGACAAACACCGGG

HSP70-1 V L I O V Y E G E R A M T K D N N L L G R F E L S G I P P A -1617
HSP70-2 GTCTGATCCAGGTGTACGAGGCGGAGGCGCATGACGAAGACACAATCTGTTGGGGGCTTCAGCTGAGCGGCATCCCTCCGGC

HSP70-1 P R G V P O I E V T F D I D A N G I L N V T A T D K S T C K -1707
HSP70-2 CCCAGGGCGTCCCGCAGATCGAGGTGACCTTGCATCGATGCCAAGGCATCTGAACGTACGGCCAGGACAAGAGCAGCGCAAG

HSP70-1 A N K I T I T N D K G R L S K E E I E R M V O E A E K Y K A -1797
HSP70-2 GCCAACAAGATCACCATCACCACGACAAGGCGCGCTGAGCAAGGAGGATGAGCGCATGTGTCAGGAGCGGAGCAAGTACAAAGG

HSP70-1 E D E V O R E R V S A K N A L E S Y A F N M K S A V E D E G -1887
HSP70-2 GAGGACGAGTGCAGCGGAGAGGCTGTGAGCAAGAACCGCTGAGCTCTACGCTTCAACATGAAGAGCGCGGTGAGGATGAGGG

HSP70-1	CTCAAGGGCAAGATCAGGAGGCGGACAAAGAAGTCTGGACAAGTCTCAAGAGCTCATCTCGTGGCTGGACGCCAACACCTTGGCC	1977
HSP70-2	CTCAAGGGCAAGATCAGGAGGCGGACAAAGAAGTCTGGACAAGTCTCAAGAGCTCATCTCGTGGCTGGACGCCAACACCTTGGCC	1975
HSP70-1	E K D E F E H K R K E L E Q V C N P I I S G L Y O G A G G P	
HSP70-2	GAGAAGGACGAGTTTGAGCACAAGAGGAGGCTGGAGCAGGTGTGTAAACCCATCATCAGCGGACTGTACCAGGCTGCCGCTGGTCCC	2067 2065
HSP70-1	G P C G F G A Q G P K G G S G S C P T I E E V D *	
HSP70-2	CGCGCTGGGGCTTCGGGCTCAGGCTCCCAAGGAGGCTCTGGTCAGGCCCCACCATTCAGGAGGTAGATTAGGGCCCTTCCAAGAT	2157 2155
HSP70-1	TGCTGTTTGTGTTTGGAGCTTCAAGACTTTCGATTCTCTACTATTCTGTTTGTGAGTTCTCAATTCCTGTGTTTCAATGTTGAAAT	2247
HSP70-2	AGTATG GTC AG TGGACT TTGGACTCAAGGACT TGC GTC T CTA G T CTGCT CAGCT TT C CTTC	2245
HSP70-1	TTTTGCTGAAGTACTGAAGTTCGCTTTTTCGGTTCACATGCAGAGATGAATTTACTGCCATCTTACGACTATTTCTCTTTT	2337
HSP70-2	AC CTT T AAGT T ACCTGA GG AA TA C GG T TA TTTTGT G AC AC GATATGT C TT GA CT GCA	2335
HSP70-1	TAATACCTTAAGTACAGGCCATTTTAAAGTTGGTACTTCAAGTAAATAAATTTAAATTCAGTGATGCCCTTTTATTCCTTTATT	2427
HSP70-2	GTGA CTGT A GGTG CGTCCCTT A A CA TCAAC C GCCAC TGTG A G TTGTTG T TT T	2425
HSP70-2	TTTTTTTTTGTCTGCCAAACACTACAAAGGCTGGAGTGTATGTTTTATAATTGTTTATTTAAATGAAAATAAATGTTAAAC	2515
HSP70-2	TTTTCTTGTCTGTAATATGTGAAGATAATGGATATTGCGGAGGGATAGTGTCTGAATACCATCTATCTTTTACTCTGAAAAGAACA	2605

Fig. 2. Comparison of the nucleotide sequences of the *HSP70-1* and *HSP70-2* genes. The upper line shows the sequence of *HSP70-1* and the lower line shows nucleotide differences in *HSP70-2*. The transcriptional start site identified by Wu and co-workers (1985) is marked by an arrow and defined as position +1. The heat shock consensus elements (GAA/TTC) are indicated by lines above the sequence. The distal and proximal CAAT homologies (ATTGG), the TATA box (TATAAAA), and the polyadenylation signals (AATAAA) for both *HSP70-1* and *HSP70-2* are underlined. The stop codon (TAG) is indicated by an asterix. The derived protein sequence which is identical for both *HSP70-1* and *HSP70-2* is shown above the nucleotide sequence.

Hunt and Morimoto (1985). Oligonucleotides taken from sequence positions 468-489, 762-781, 1084-1101, 1342-1360, 6122-6149, and 1886-1904 (see Fig. 2) were used as sequence primers for the coding regions of both the *HSP70-1* and *HSP70-2* genes. In addition, oligos from sequence positions 2120-2137 in *HSP70-1* and positions 2118-2135 in *HSP70-2* were used to sequence the 3' untranslated regions.

Southern blot analysis of genomic DNA. Genomic DNA (5 µg) was digested with the appropriate restriction enzyme using the conditions recommended by the supplier (Amersham, Amersham, UK). The digested DNA was fractionated on a 0.8% agarose gel, transferred to nitrocellulose (Southern 1975), and hybridized with ³²P-labeled probes. Probes were either labeled directly in LGT agarose, or DNA was purified using GeneClean (Stratagene Scientific, London, UK) prior to labeling by random hexanucleotide priming (Feinberg and Vogelstein 1984). Blots were hybridized for 24 h at 42 °C in 50% formamide/5 × Denhardt's solution/10% dextran sulfate/1 M NaCl/50 mM Tris-HCl pH 7.4/0.1% sodium dodecyl sulfate (SDS) containing 100 µg/ml sonicated salmon sperm DNA. High-stringency washes were performed at 65 °C in 0.1 × standard sodium citrate (SSC)/0.1% SDS for 1 h. Blots were autoradiographed between two intensifying screens at -70 °C for 1-5 days.

Isolation of RNA and northern blot analysis. HeLa and U937 cells were grown in tissue culture to densities of 1-2 × 10⁶ cells/ml. Cells were either maintained at 37 °C or heat-shocked at 42 °C for 2 h prior to their collection by centrifugation. Some HeLa cells were also fed with 2-deoxy-D-glucose at a final concentration of 10 mM for 12 h or starved of serum, by transfer to serum-free media for 24 h, prior to their collection. Total RNA was extracted by guanidinium isothiocyanate lysis and caesium chloride ultracentrifugation (Chirgwin et al. 1979; Maniatis et al. 1982). Polyadenylated poly(A)⁺ mRNA was isolated from the total RNA by oligodeoxythymidylate chromatography (Maniatis et al. 1982). Samples of total RNA (15 µg) or 2 µg samples of poly(A)⁺ RNA were fractionated in 0.8% agarose-formaldehyde denaturing gels and transferred onto nitrocellulose (Fourney et al. 1988). Northern blots were hybridized with ³²P-labeled probes under the same conditions as genomic Southern blots, as outlined above. High-stringency washing was carried out at 65 °C in 0.2 × SSC/0.1% SDS for 1 h, prior to autoradiography at -70 °C between intensifying screens for 2-10 days.

Results

The *HSP70-1* and *-2* genes. The complete sequences of the *HSP70-1* and *HSP70-2* genes and their 5' flanking sequences were determined as described in Materials and methods. The *HSP70-1* gene has an open-reading frame of 1923 base pairs (bp) from the ATG codon at nucleotide 217 to the stop codon (TAG) at nucleotide 2140 (Fig. 2). The predicted relative mass of *HSP70-1* from the derived protein sequence (641 amino acid residues) is 70053. In the 3' untranslated region the polyadenylation signal AATAAA lies 242 bp from the stop codon. Comparison of the sequence shown in Figure 2 with the previously published *HSP70* gene sequence (Hunt and Morimoto 1985) revealed 11 nucleotide differences. Two of these differences, a C-G transversion at nucleotide 190 and a G insertion at nucleotide 215, lie in the 5' untranslated region. The remaining nine differences lie within the coding sequence. Of these, two single-base changes predict changes in the derived amino acid sequence at position 7 (GTC-ATC; Val-Ile) and at position 371 (GGC-GCC; Gly-Ala), in agreement with the results of Sargent and co-workers (1989b). The presence of three single-base insertions at nucleotides 1620, 1622, and 1623 in our sequence predicts an extra codon at position 1621. The sequence of nucleotides 1618-1626 in Figure 2 is 5'CCCAGGGGC3' and encodes Pro-Arg-Gly. The sequence of Hunt and Morimoto (1985) is 5'CCAGGC3' and encodes Pro-Gly. The additional Arg in our sequence is also found in *Drosophila HSP70*, *E. coli dnaK*, and *HSP70B'* (Fig. 4). The differences between our sequence and that determined by Hunt and Morimoto (1985) could represent allelic polymorphism in the *HSP70-1* gene.

The *HSP70-2* sequence contains a continuous open-reading frame extending from nucleotides 215 to 2138 (Fig. 2), and like the *HSP70-1* gene lacks introns. The derived protein sequence of 641 amino acid residues is identical to that of *HSP70-1*. The eight nucleotide differences at positions 436, 799, 802, 1267, 1923, 2074, 2119, and 2134 (nucleotide positions refer to *HSP70-2*) lie in the second or third base of a codon and do not cause alteration in the derived amino acid sequence (see Fig. 2). The G→A transition at position 1267 in *HSP70-2* results in the loss of a *Pst* I restriction site. This is the basis for the *Pst* I polymorphism defined by Goate and co-workers (1987). In the 3' untranslated region of *HSP70-2* the polyadenylation signal lies 361 bp from the stop codon. Comparison of the 3' untranslated region of the *HSP70-1* gene with the equivalent region of the *HSP70-2* gene reveals that they are completely divergent (Fig. 2).

The 5' untranslated region of the *HSP70-1* gene is 217 bp and is identical to that determined by Hunt and Morimoto (1985) except for the two single-base changes mentioned above. Although the transcriptional start site of the *HSP70-2* gene has not been determined, it is likely that it is at a similar position to that of the *HSP70-1* gene. In primer extension experiments reported by others (Wu et al. 1985), only a single extended product was defined. Given that both genes are transcribed into abundant mRNA species after heat shock (Fig. 6) and that the two sequences differ by only 26 single-base changes, any primer extension based on *HSP70-1* mRNA with the primers used would also have occurred on *HSP70-2* mRNA. In addition, this would place the TATA box sequence at the appropriate place in the *HSP70-2* gene from the transcriptional start site.

Comparison of the 5' flanking sequence of the *HSP70-1* and -2 genes reveals an additional 17 single-base changes extending back as far as nucleotide -273. However, none of the differences seriously disrupt any of the elements so far identified in the *HSP70-1* gene as being important for the basal level of transcription of the gene, or for transcriptional activation after stress (Wu et al. 1986; Morgan et al. 1987; Amin et al. 1988; Williams et al. 1989; Taylor and Kingston 1990).

The homologous region. The 0.9 kb *Bgl* II probe (H), derived from the *HSP70-1* gene, in addition to hybridizing to the *HSP70-1* and *HSP70-2* genes on Southern blot analysis, was found to hybridize less strongly to a region lying ~4 kb telomeric to the *HSP70-1* gene (Sargent et al. 1989b). In order to define the structure of the homologous region the sequence of 3330 bp of DNA was determined (Fig. 3). This indicated that the homologous region represents another gene (*HSP70-Hom*) of the *HSP70* family. The *HSP70-Hom* gene contains an open-reading frame of 1923 bp from the ATG codon at

nucleotide 960 to the stop codon (TAA) at nucleotide 2883. Like the *HSP70-1* and -2 genes, it lacks introns. The 3' untranslated sequence contains a polyadenylation signal ATTAAA 405 bp from the stop codon and differs significantly from that of the *HSP70-1* and -2 genes. Upstream of the ATG codon at nucleotide 960 are two Alu repeat sequences between nucleotides 294 and 900. A TATA box is found at nucleotide 162. However, no CAAT box sequence or heat shock consensus element with GAA/TTC repeats is found up to 959 bp from the ATG codon.

Comparison of the amino acid sequences of *HSP70-Hom* and *HSP70-1* (Fig. 4) reveals that they are 90% similar. If conservative replacements are taken into account the sequence similarity increases to 94%. The greatest sequence divergence between these two proteins is at the C terminus. The similarity in the C-terminal 100 amino acids is only 72%. There is also a high level of protein sequence identity between *HSP70-Hom* and *HSP70-B'* (79%; Leung et al. 1990) and between *HSP70-Hom* and *HSC70* (84%; Dworniczak and Mirault 1987). Again the sequences differ most in the C-terminal 100 amino acids, where the percentage similarity decreases to 47% between *HSP70-Hom* and *HSP70B'*, and to 64% between *HSP70-Hom* and *HSC70*.

Southern blot analysis. The human genome contains multiple copies of expressed genes and unexpressed pseudogenes in the *HSP70* family. Due to the high degree of sequence similarity between the coding regions of the *HSP70* genes, DNA probes corresponding to coding regions tend to cross-hybridize to each other. This is apparent in Figure 5A where probe H, in addition to hybridizing to the 2.4 kb, 5 kb, and 7 kb *Bam* HI/*Hind* III fragments which correspond to the *HSP70-1*, *HSP70-Hom*, and *HSP70-2* genes, respectively, also hybridized to other fragments corresponding to *HSP70* genes elsewhere in the genome. In order to differentiate between the various genes in genomic Southern blot analysis and to provide evidence for the expression of the MHC-linked *HSP70* genes in northern blot analysis, it was necessary to define probes corresponding to the regions of minimum sequence similarity between the various genes. Given that the 3' untranslated regions of each of the three genes showed little sequence similarity to each other or to the 3' untranslated regions of the *HSP70B'* (Leung et al. 1990) and *HSC70* (Dworniczak and Mirault 1987) genes, the isolation of suitable locus-specific probes was possible.

A 510 bp *Eco* 0109/*Hind* III fragment, a 600 bp *Eco* 0109 fragment, and a 225 bp *Eco* 0109 fragment were isolated from the 3' untranslated regions of the *HSP70-1*, *HSP70-2*, and *HSP70-Hom* genes, respectively. Each of these probes was hybridized to a Southern blot of genomic

GCAGCTATGACCTGGGAGGTGAGGAC* TGAGCCATGATTACACCACTGCAGTGCAGCCTGCCTGACAAAACGAGACCT 90
 TAAAAATGACAAAAAAATGGTGTGTACACGGCGATAAGGGAGGGGAAAAACGGAGTACTTAATGAGTATACAGTTTCAGTTTGC 180
 GAGATGAACAGAAATCTGGAAATGGTTGAACACCGCTGCTGATTGAACCTCACTACCAACTCTACACTTAAAAATGGTTAAGTGGTACA 270
 ATTTGTATGTATTTTACCACAATAAAAAATAAAAAAGGCTGGGGAGATGTTACCTCTGTAATCCAGTACTTGGGGAGGCTGGGGC 360
 TGAAGGATCGTTTGAGCCCTGAAGGAGTTTGAGACACAGCTGACCAACATAAGGAGACCCATCTGTACACAAAATTAACATTAGCCA 450
 GGCAGAGAGCTGGTCAAGGGGCTCAGTATGTAATCCAGCACTTGGGAGGCGGAGCGGGCGGGGATCACCTGAGGTGAGGAGTT 540
 TGACACACGCTGGCCACATAGTGAACCGTGAAACCCCATCTCTACTAAAAATACAAAAATTAGCTGGGCGTGGTGGTCCCTCATAA 630
 TCCACGCACTCGGAGGCTGACACAGGAGATCGCTTGAACCTAGGAGTGGAGTTGCAGTGAGCTAGATCACACCACTGCAGTCCA 720
 AAGCAAGACTCCGCTCAAAAAATAAAAAATAGCCCGGCTGTGTCTCCAGTTATTCTGGAGGCTAAGGCAGGAAGATTGCTGGAGCC 810
 TAGGAGATCAAAAGCTGCAGTGAGCTATGACTGGGCTCTGCACTCAACCTGGGTGACAGGAAGACCTGTCTCAAAAAATAAATAA 900
 CATTGAAGGAAGCTCTCCCAAAAGTATCTTATTCTTCTCATAGGCTCAGAGAACCATGGCTACTGCCAAGGAATCGCCATAGGAA 990
 IDLGTITYSCVGVFOH GKVEIIANDOGNRTT
 TCGACCTGGGCACCACCTACTCCTGTGTGGGGTGTCCAGCACGGCAAGGTGGAGATCATCGCAACGACAGGGAACCGCACCC 1080
 PSYVAFTDTERLIGDAAKNOVAMNPONTVF
 CAGCTACGTGGCTTCAGAGACACCGGCTCATGGGATGCGGCAAGACAGGTAGCAATGAATCCCGAGACACTGTTTTG 1170
 DAKRLIGRKFNDPVVQADHKLWPFQVINEG
 ATGCTAAAGCTGTGATCGGACGAAATTAATGATCCTGTGTACAGCAGATGAAACTTTGGCTTTTCAAGTATTAATGAAGGAG 1260
 GKPKVLVLSYKGENKAFYPEEISSMVLTKLK
 CCAAGCCAAAGCTGTGTCTACAAAGGGAGATAAAGCTTCTACCTCAGGGAATCTCTTCGATGGTATTGACTAAGTTGAAGG 1350
 ETAEAF LGHPVTNAVITVPAYFND SORQAT
 AACTGTGTGGGCTTTTGGCCACCTGTCAACATGCACTGATTACCGTGGCAGGCTATTTCACACTCTCAACGTCAGGCTACTA 1440
 KDAGVIA GLNVLRIINEPTAAAIAYGLDKG
 AGGATGCGGTGTGCTGGACTTAATGTGCTAAGAAATCATCAATGAGCCACGGCTGTGCGCATGGCTATTGATTAAGGAG 1530
 CGGERHVLIFDLGGCTFDVSI LTI DDGIFE
 GTCAGGAGAACGACATGTCTGATTTTGATCTGGGTGGAGGACATTTGATGTGCAATTTCAGCAGTATGATGGGATTTTGAGG 1620
 VKATAGDTHLGGGEDFDNRLVSHFVEEFKRK
 TAAAGCCACTGCTGGGACACTACCTGGGTGGGAGGACTTGCACACAGGCTTGTGAGCCACTTGTGGAGGAGTTCAAGAGGAAAC 1710
 HKKDISONKRAVRRLRTACERAKRTLSSST
 ACAAAAGGACATCAGCCAGAACAGCGAGCGGTGAGCGGCTGCGCACCGCTGCGAGAGGGCCAAAGAGGACCTGCTGCTCCAGCACC 1800
 QANLEIDSLYEGIDFYTSITRARFEELCAD
 AGGCCAAGCTAGAAATGATTCACTTTATGAAGCATTGACTTCTATACATCCATCAGCAGGCTCGATTGAAGAGTTGTGTGAGAGC 1890
 LFRGTLLEPDKALRDADKAKINDIVLVG
 TGTTTAGGGTACCCTGGAGCTGTAGAAAAAGCGCTTCGGGATGCCAAGATGAGTAAGGCTAAATCCATGACATTGTTTATGAGGG 1980
 GSTRI PKVORLLQDYFN GRDLNKSINPDEA
 GCTCCACCGCATCCCAAGGTGAGCGGCTGCTTCAGGACTACTCAATGGAGCTGATCTCAACAGAGCATCAACCTGATGAGGCGG 2070
 VAYGAAVQAAILHGD KSEKVO D L L L L D V A P
 TAGCATATGGGCTGCGGTACAAGCAGCATCTGTATGGGGGACAGTCTGAGAAGGTACAGGACCTGCTGCTGCTGGAGCTGGCTCC 2160
 LSLGLELTVGGVM TALIKR NSTIPP KOT O I F
 TGTCCCTGGGTGAGAGCGGTTGGGGGCTGATGACTGCCCTGATAAAGCGCAACTCCACCATCCACCCAAAGCAGACACAGATTTCA 2250
 TTYSDNOPGVLIQVYEGERAHTKDN N L L GR
 CCACCTACTCTGACACCAACCCGGGTGCTGATCCAGGTGTATGAGGGGAGAGGGCCATGACAAAGGACAAACCTGCTGGGGCGGT 2340
 FDLTGIPPA PRGVPOIEVTFDIDANGILNV
 TTGATCTGACTGGAATCCCTCCAGCACCCAGGGAGTCTCTCAGATCGAGGTGACGTTTGACATTGATGCCAATGGTATTCTCAATGTCA 2430
 TATDKSTGKVNK I T I T N D K G R L S K E E I E R M
 CAGCCACGGACAGAGCAGCGGCAAGGTGAACAGATCACCATCACCATGACAAGGGCGGCTGAGCAAGGAGGAGATTGAGCGGATGG 2520
 VLD AEKYKA EDEVOREKIAAKNALESYAFN
 TTCTGGATCTGAGAAATAAAGCTGAAGTGAAGTCCAGAGGAGAAATGCTGCAAAAGTGCCTTGAATCCTATGCTTTAACA 2610
 M K S V V S D E G L K I S E S D K N K I L D K C N E L L
 TGAAGAGTGTGTGAGTGTAAGCTTTGAAGGCAAGATTAGTGAGTCTGATAAAAAATAAATAATTGGATAATGCACAGGCTCTTT 2700
 S W L E V N O L A E K D E F D H K R K E L E O M C H P I I T
 CGTGGCTGGAGTCAATCAACTGCCAGAGAAAGATGAGTTGATCATAAGAGAAAGGAATTGGAGCAGATGTGTAACCTATCATCACA 2790
 K L Y O G G C T G P A C G T G Y V P G R P A T G P T I E E V
 AACTCTACCAAGGAGGATGCACTGGCCCTGCTCGGAACAGGTATGTGCTGGAAGGCTGCCACAGGCCCAACAATTGAAGAGTAG 2880
 D *
 ATTAATTTCTTTTGAAGTGAAGCATCTAGGATGCTCTACATGTATTTCATTCCTCATGTTGAAACATCATTATTATTCTTGACCA 2970
 CACCTGAATCTAAGTTACCATCCCTTGAATCTGGAGAAGGAGTCTCATGCACCACTATCACACTCCCTCACATCTGTTTCTGACT 3060
 TTGAATGGACTCAGGAAAAC TAGGCCCTCTTAAACGCTGATGATTGAATGTCTGTTATTTCCAGCCACCTAACATTCTTCTTC 3150
 CTCTGTGATGCTTATTGTCATCAGTAAATTTGTTCTGAAGAAAATTAATCTCTGTTATTTAGGCTGTGAATGACCTTGAAGGGAG 3240
 AACTCATGGAGAGCATGTCTCTGCTGATGAGGTCACTGTAATCATATAATTGGTAAGGCTAAAGTATTGCAATTTTCATGAAGT 3330

Fig. 3. Sequence of the *HSP70-Hom* gene. The TATA box and polyadenylation signal (ATTAAG) are underlined. The stop codon (TAA) is indicated by an asterisk. The derived protein sequence is shown above the nucleotide sequence.

HSP70-1	MA--KAAAIIGIDLCITYSCVGVFOHCKVEIIANDOGNRTIPSYVAFTÖTERLIGD	53
HOM	M.TA.GI.....O.R.....L.....V.....	55
HSP70-B'	MOAPREL.V.....O.R.....L.....V.....	55
HSC70	MS--GP.....	53
HSP70-1	AAKNQVAI.NPONTV.FDAKRLIGRKFGDPVVQSDMKHWPFOVINDGDKPKVOVSYK	106
HOM	..S.A.....H.....A.TT.....R.VSE.C.....L.....	110
HSP70-B'	..S.A.....H.....A.TT.....R.VSE.C.....L.....	110
HSC70	..M.T.....R.D.A.....H.....VSE.AGR.....P.....R.....	108
HSP70-1	GETKAFYPEEISSMVLTKMKIEAEAYLGYPVTNAVITVPAYFNDSSOROATKDAKV	163
HOM	..N.....L.T.....F.....H.....	165
HSP70-B'	..D.T.....S.....T.....O.KH.....	165
HSC70	..S.....V.....T.....KT.....V.....	163
HSP70-1	IAGLNVLR.IINEPTAAAIAYGLDRT-CKGERNVLI.FDLGGCTFDVSILTI.DDGIF	217
HOM	..K.G.....O.....H.....	219
HSP70-B'	..K.G.....O.....H.....	219
HSC70	..K.KV.....V.S.A.V.....E.....	218
HSP70-1	EVKATAGDTHLGGEDFDNRLVNH.FVEEFKRKHKKDISONKRAVRRLRTACERAKR	272
HOM	..S.....M.....R.....G.....L.G.....L.G.....	274
HSP70-B'	..S.....M.....R.....G.....L.G.....L.G.....	274
HSC70	..S.....M.....R.....G.....L.G.....L.G.....	273
HSP70-1	TLSSSTOASLEIDSLFEGIDFYTS:TRARFEELCSCLFRSTLEPVEKALRDAKLD	327
HOM	..K.....Y.....V.....A.....G.....M.....	329
HSP70-B'	..K.....Y.....V.....A.....G.....M.....	329
HSC70	..K.....Y.....V.....A.....G.....M.....	328
HSP70-1	KAO:HDVLVLVGGSTRIPKVKOKLLOCFENGRLNKSINPDEAVAYCAAVOAAIILKC	383
HOM	..K.....R.....Y.....KE.....V.....S.....	385
HSP70-B'	..K.....R.....Y.....KE.....V.....S.....	385
HSC70	..S.....I.....I.....KE.....	384
HSP70-1	DKSENVQDL.LLLDVAPLSLGLLETAGCVMTALIKRNSTIPTKOTDIFTTYSKOPG	437
HOM	..K.....V.....T.....O.....A.....P.....T.....	439
HSP70-B'	..K.....V.....T.....O.....A.....P.....T.....	439
HSC70	..K.....V.....T.....O.....A.....P.....T.....	438
HSP70-1	VLIQVYEGERAKTKDNKLLGRFELSGIPAPRGVPOIEVTFDIDANGILNVTATD	492
HOM	..F.....C.....K.....T.....S.....S.....V.....	494
HSP70-B'	..F.....C.....K.....T.....S.....S.....V.....	494
HSC70	..F.....C.....K.....T.....S.....S.....V.....	493
HSP70-1	KSTGKAKKKTITTKDKGRLSKEEIERMVQEAEEKYKAECDEVGRERVSAKNALESYAF	547
HOM	..V.....S.....N.....H.....O.....A.....D.....K!A.....S.....AHV.....	549
HSP70-B'	..V.....S.....N.....H.....O.....A.....D.....K!A.....S.....AHV.....	549
HSC70	..E.....D.....K.....K.....S.....S.....	548
HSP70-1	NKMSAVEDEGLKGGK:SEACKKKVLCKCCEVISWLDANTLAEKDEFHKKRKELEOV	602
HOM	..V.....S.....N.....H.....O.....A.....D.....K!A.....S.....AHV.....	604
HSP70-B'	..V.....S.....N.....H.....O.....A.....D.....K!A.....S.....AHV.....	604
HSC70	..V.....S.....N.....H.....O.....A.....D.....K!A.....S.....AHV.....	604
HSP70-1	CKPIISGLY-----OGA-CGPGPG-GFGAOGPKGGSGSGPTIEEV	641
HOM	..TK.....CCT.....AC.T.YVP-----RPA.....	641
HSP70-B'	..TK.....CCT.....AC.T.YVP-----RPA.....	641
HSC70	..TK.....OSAGGPGGGK-GF-PG.CAP.S.S.A.S.....	642
	..TK.....OSAGGPGGGK-GF-PG.CAP.S.S.A.S.....	641

Fig. 4. Comparison of the protein sequences of HSP70-1 and HSP70-Hom with those of the stress-induced protein HSP70-B' (Leung et al. 1990) and the constitutive protein HSC70 (Dworkiczak and Mirault 1987). Amino acid residues that differ from HSP70-1 are shown and identical amino acids are indicated by a dot. Dashes are inserted to optimize alignment of the protein sequences.

DNA digested with *Eco* RI, *Bgl* II, *Bam* HI, and *Hind* III in single- and double-digest combinations. In all cases the probes hybridized uniquely to fragments associated with the *HSP70* locus from which they had been isolated, and to no other fragments (Fig. 5B).

Northern blot analysis. The locus-specific probes described above were used to examine the expression of the MHC-linked *HSP70* genes. Northern blots were prepared using total RNA from HeLa cells either maintained at 37 °C, heat-shocked for 2 h at 42 °C, fed with 2-deoxy-D-glucose for 12 h, or serum-starved for 24 h prior to harvesting. In addition, poly(A)⁺ RNA from control and heat-shocked U937 cells was used.

With both the *HSP70-1* and *HSP70-2* locus-specific probes an elevated level of a ~2.4 kb mRNA was observed from the heat-shocked HeLa (Fig. 6) and U937 cells (data not shown). A very low level of *HSP70-1* mRNA

was detectable in the control cells, but this was not observed for *HSP70-2* (Fig. 6). However, neither 2-deoxy-D-glucose treatment nor serum starvation had any apparent effect on the level of message observed constitutively, in agreement with the results of others (Wu and Morimoto 1985; Wu et al. 1985; Watowich and Morimoto 1988). Assuming that the *HSP70-1* and *HSP70-2* genes share the same transcriptional start site (defined by Wu et al. 1985), the positions of the polyadenylation signals in these two genes predict an mRNA for *HSP70-2* ~100 bp longer than that for *HSP70-1*. The resolution available by northern blotting is not sufficient for this difference to be seen.

When the *HSP70-Hom* locus-specific probe was used to probe northern blots a very low level of an mRNA of ~3 kb was detected from control HeLa cells (Fig. 6) and also heat-shocked, 2-deoxy-D-glucose-treated, and serum-starved cells, a result which indicates that this gene is

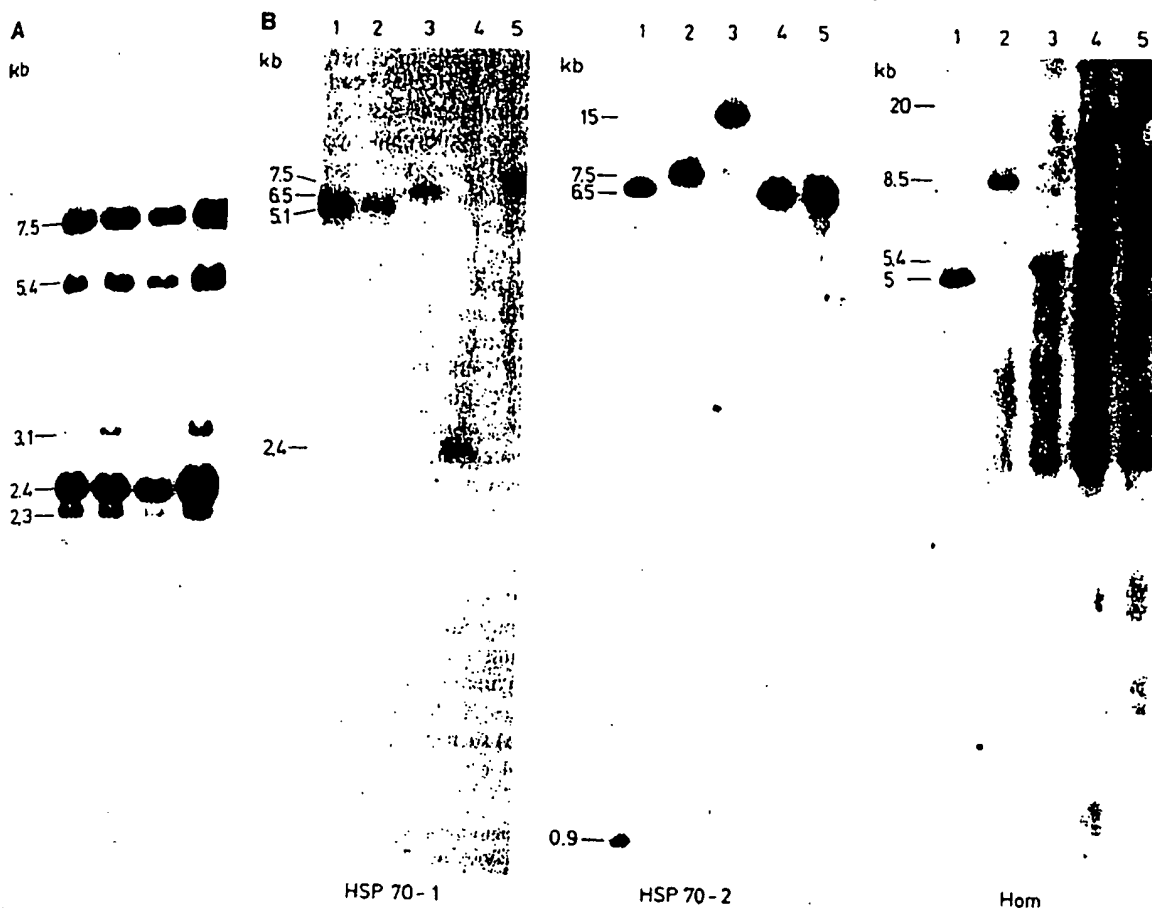


Fig. 5. A, B. Southern blot analysis. A A Southern blot of *Bam* HI/*Hind* III-digested genomic DNA (5 µg) from four individuals was hybridized with the ³²P-labeled 0.9 kb *Bgl* II probe (H). After high-stringency washing and autoradiography the probe was found to hybridize strongly to fragments of 7.5, 5.4, and 2.4 kb, which correspond to the MHC-linked *HSP70* loci. In addition, probe H hybridized less strongly to fragments of 3.1 and 2.3 kb, associated with *HSP70* loci elsewhere in the genome. B Locus-specific probes for the MHC-linked *HSP70* loci. Southern blots of genomic DNA (5 µg) from an *HLA*-homozygous cell line (ICE 5) digested with *Eco* RI/*Bgl* II (1), *Bgl* II (2), *Hind* III (3), *Bam* HI/*Hind* III (4), and *Bam* HI (5) were hybridized with the ³²P-labeled locus-specific probes prepared from the 3' untranslated regions of the MHC-linked *HSP70* genes. After high-stringency washing and autoradiography, each of the probes hybridized only to fragments shown by cosmid mapping to be associated with the genes from which they had been derived. Sizes of fragments are shown on the left of each blot.

expressed constitutively at a low level but is not induced by heat shock, 2-deoxy-D-glucose, or serum starvation. Although the transcriptional start site of the homologous gene has not been determined, an mRNA of ~3 kb is consistent with the positions of the TATA box (nucleotide 162) and polyadenylation signal (nucleotide 3290).

Discussion

Our analysis of the MHC-linked *HSP70* loci has shown the presence of two intronless genes, *HSP70-1* and *HSP70-2*, which encode an identical protein product and which both express elevated levels of a ~2.4 kb mRNA following heat shock. The observation that two closely linked *HSP70* loci both express an identical protein is somewhat surprising. One possible explanation is that the

range of stress factors which induce *HSP70* expression is too great for the promotor sequence of a single gene to recognize them all. Therefore, two genes with different 5' promotor sequences, as in the case of *HSP70-1* and *HSP70-2*, are required to provide an adequate response. In addition, there may be differences in the expression of the two genes in different cell types due to the sequence differences in the promoter regions. Also, the sequences in the 3' untranslated region which are completely divergent between *HSP70-1* and *HSP70-2* may confer different regulation on the two mRNA species.

Lying ~4 kb telomeric of *HSP70-1* is a third intronless gene, *HSP70-Hom*, which encodes a protein with 90% similarity to *HSP70-1*. The *HSP70-Hom* gene appears to be constitutively expressed, but levels of its mRNA are not increased following heat shock. This novel

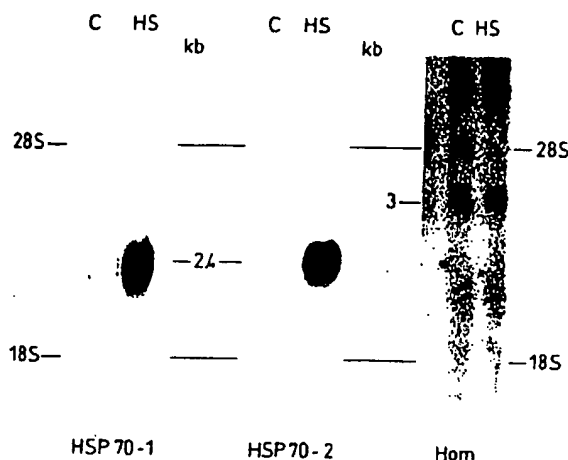


Fig. 6. Northern blot analysis. Total cytoplasmic RNA (15 µg) derived from HeLa cells, either maintained at 37 °C or heat-shocked for 2 h at 42 °C prior to harvesting, was fractionated on 1% agarose-formaldehyde denaturing gels and transferred onto nitrocellulose to give three identical blots. The northern blots were hybridized with the three *HSP70* locus-specific probes. The position of migration of the 28S and 18S RNA is shown. The *HSP70-1* probe detected a transcript of ~2.4 kb at a very low level in constitutive RNA (C) and at a much elevated level in heat-shocked RNA (HS). The *HSP70-2* probe detected no transcript in constitutive RNA (C), and a high level of a ~2.4 kb transcript in heat-shocked RNA (HS). The homologous gene probe detected low levels of a ~3 kb transcript in constitutive (C) and heat-shocked (HS) RNA.

HSP70 gene encodes an *HSP70* variant that is more basic than *HSP70-1*, and is distinct from *HSC70* and the recently described *HSP70B'* (Leung et al. 1990), which are encoded by genes in other parts of the genome.

Comparison of *HSP70-1* and *HSP70-Hom* with other members of the *HSP70* protein family reveals that they are highly similar (79–84%). The greatest divergence is in the C-terminal 100 amino acids (*HSP70-Hom* vs *HSP70-1*, 72%; *HSP70-Hom* vs *HSP70B'*, 47%; *HSP70-Hom* vs *HSC70*, 64%) and this may indicate a selective function of the different *HSP70* proteins.

Transcription of the *HSP70-1* gene has been shown to be induced by serum stimulation after serum starvation (Wu and Morimoto 1985). However, serum withdrawal alone has been shown to result in increased levels of a cytosolic protein, prp 73, in rats (Chiang et al. 1989). This protein has been shown to be a member of the *HSP70* family and has been found to bind to peptide regions that target intracellular proteins for lysosomal degradation, in response to serum withdrawal. We therefore looked for elevated levels of mRNA from the MHC-linked *HSP70* genes following serum withdrawal, but this was not observed. Elevated levels of mRNA from the glucose-responsive *HSP70* family member *GRP78* (*BiP*) have been shown to occur following treatment with 2-deoxy-D-glucose (Watowich and Morimoto 1988). However, elevated levels of mRNA were not detected for any of the

MHC-linked *HSP70*, following 2-deoxy-D-glucose treatment of cells in culture.

The MHC has been shown to influence more than 40 autoimmune diseases. In some cases, a direct involvement of the polymorphic class I and class II and the less variable class III gene products has been proposed (Batchelor and McMichael 1987; Todd et al. 1988). In many cases, however, the role of the MHC is unclear. There is a great deal of interest in the possible roles that stress proteins may play in immune responses (for review see Young 1990), and there is evidence to suggest that *HSP70* proteins may be involved in autoimmune diseases (van Eden et al. 1988; Res et al. 1988; Lamb et al. 1989; Yasuhiro and Kishimoro 1990). However, due to the very high degree of sequence similarity it is not easy to distinguish between the different *HSP70* family members using cDNA or genomic probes. Thus, the locus-specific probes reported here will make it possible to investigate variations in gene copy number and variations in the levels of expression of the MHC-linked *HSP70* genes in relation to those *HLA* types frequently associated with autoimmunity. In addition, the sequence differences in the 5' and 3' untranslated regions allow the specific amplification by polymerase chain reaction of the MHC-linked *HSP70* genes (C. M. Milner and R. D. Campbell, unpublished data) such that sequence variation in these genes between haplotypes can be investigated.

Acknowledgments. We wish to thank Alison Marsland, Ken Johnson, and Tony Day for their help in preparing the manuscript and Dr. Ken Reid for his critical review of the text. We sincerely thank Tony Day for oligonucleotide synthesis, Carole Sargent for cosmid H92 and I81, and Sandra Smith for technical assistance. C.M.M. holds a Medical Research Council Studentship.

References

- Amin, J., Ananthan, J., and Voellmy, R.: Key features of heat shock regulatory elements. *Mol Cell Biol* 8: 3761–3769, 1988
- Batchelor, J. R. and McMichael, A. J.: Progress in understanding HLA and disease associations. *Br Med Bull* 43: 156–183, 1987
- Cameron, P. U., Tabarias, H. A., Pulendran, B., Robinson, W., and Dawkins, R. L.: Conservation of the central MHC genome: PFGE mapping and RFLP analysis of complement, *HSP70*, and *TNF* genes in the goat. *Immunogenetics* 31: 253–264, 1990
- Chappel, T. G., Welch, W. J., Schlossman, D. M., Palter, K. B., Schlesinger, M. J., and Rothman, J. E.: Uncoating ATPase is a member of the 70 kilodalton family of stress proteins. *Cell* 45: 3–13, 1986
- Chiang, H. L., Terlecky, S. R., Plant, C. P., and Dice, J. F.: A role for a 70 kilodalton heat shock protein in lysosomal degradation of intracellular proteins. *Science* 246: 382–385, 1989
- Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J.: Isolation of biologically active RNA from sources enriched in ribonuclease. *Biochemistry* 18: 5294–5299, 1979
- Dworniczak, B. and Mirault, M.: Structure and expression of a human gene coding for a 71 kD heat shock 'cognate' protein. *Nucleic Acids Res* 15: 5181–5197, 1987
- Feinberg, A. P. and Vogelstein, B.: A technique for radiolabelling DNA

- restriction endonuclease fragments to high specific activity. (Addendum.) *Anal Biochem* 137: 266-267, 1984
- Fourney, R. M., Miyukoshi, J., Day, R. F., and Paterson, M. C.: Northern blotting: efficient RNA staining and transfer. *Focus* 10: 5-7, 1988
- Guate, A. M., Cooper, D. N., Hall, C., Leung, T. K. C., Solomon, E., and Lim, L.: Localisation of a human heat-shock HSP70 gene sequence to chromosome 6 and detection of two other loci by somatic-cell hybrid and restriction fragment length polymorphism analysis. *Hum Genet* 75: 123-128, 1987
- Harrison, G. S., Drabkin, H. A., Kao, F. T., Hartz, J., Hart, I. M., Chu, E. H. Y., Wu, B. J., and Morimoto, R. I.: Chromosomal location of the human genes encoding major heat-shock protein HSP70. *Somatic Cell Mol Genet* 13: 119-130, 1987
- Hunt, C. and Morimoto, R. I.: Conserved features of eukaryotic HSP70 genes revealed by comparison with the nucleotide sequence of human HSP70. *Proc Natl Acad Sci USA* 82: 6455-6459, 1985
- Lamb, J. R., Bal, V., Mendez-Samperio, P., Mehleri, A., So, A., Rothbard, J., Jindal, S., Young, R. A., and Young, D. B.: Stress proteins may provide a link between the immune response to infection and autoimmunity. *Int Immunol* 1: 191-196, 1989
- Leung, T. K. C., Rajendran, M. Y., Monfries, C., Hall, C., and Lim, L.: The human heat-shock protein family. *Biochem J* 267: 125-132, 1990
- Levi-Strauss, M., Carroll, M. C., Steinmetz, M., and Meo, T.: A previously undetected MHC gene with an unusual periodic structure. *Science* 240: 201-204, 1988
- Lindquist, S.: The heat-shock response. *Annu Rev Biochem* 55: 1151-1191, 1986
- Lindquist, S. and Craig, E. A.: The heat-shock proteins. *Annu Rev Genet* 22: 631-677, 1988
- Maniatis, T., Fritsch, E. F., and Sambrook, L.: *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982
- Morel, Y., Bristow, J., Gitelman, S. E., and Miller, W. L.: Transcript encoded on the opposite strand of the human steroid 21-hydroxylase/complement component C4 gene locus. *Proc Natl Acad Sci USA* 86: 6582-6586, 1989
- Morgan, W. D., Williams, G. T., Morimoto, R. I., Greene, J., Kingston, R. E., and Tjian, R.: Two transcriptional activators, CCAAT-box-binding transcription factor and heat shock transcription factor, interact with a human HSP70 gene promoter. *Mol Cell Biol* 7: 1129-1138, 1987
- Res, P. C. M., Schaar, C. G., Breedveld, F. C., van Eden, W., van Embden, J. D. A., Cohen, I. R., and de Vries, R. R. P.: Synovial fluid T cell reactivity against the 65 kD heat-shock protein of mycobacteria in early onset of chronic arthritis. *Lancet* 2: 478-480, 1988
- Sargent, C. A., Dunham, I., and Campbell, R. D.: Identification of multiple HTF-island associated genes in the human major histocompatibility complex class III region. *EMBO J* 8: 2305-2312, 1989a
- Sargent, C. A., Dunham, I., Trowsdale, J., and Campbell, R. D.: Human major histocompatibility complex contains genes for the major heat shock protein HSP70. *Proc Natl Acad Sci USA* 86: 1968-1972, 1989b
- Southern, E. M.: Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98: 503-517, 1975
- Spies, T., Breshahan, M., and Strominger, J. L.: Human major histocompatibility complex contains a minimum of 19 genes between the complement cluster and HLA-B. *Proc Natl Acad Sci USA* 86: 8955-8958, 1989
- Taylor, I. C. A. and Kingston, R. E.: Factor substitution in a human HSP70 gene promoter: TATA-dependent and TATA-independent interactions. *Mol Cell Biol* 10: 165-175, 1990
- Ting, J. and Lee, S. A.: Human gene encoding the 78,000-Dalton glucose-regulated protein and its pseudogene: structure, conservation, and regulation. *DNA* 7: 275-286, 1988
- Todd, J. A., Acha-Orbea, H., Bell, J. I., Chao, N., Fronck, Z., Jacob, C. O., McDermott, M., Sinha, A. A., Timmerman, L., Steinman, L., and McDewitt, H. O.: A molecular basis for MHC class II-associated autoimmunity. *Science* 240: 1003-1009, 1988
- van Eden, W., Thole, J. E. R., van der Zee, R., Noordzij, A., van Embden, J. D. A., Hensen, E. J., and Cohen, I. R.: Cloning of the mycobacterial epitope recognised by T lymphocytes in adjuvant arthritis. *Nature* 331: 171-173, 1988
- Watowich, S. S. and Morimoto, R. I.: Complex regulation of heat-shock- and glucose-responsive genes in human cells. *Mol Cell Biol* 8: 393-405, 1988
- Williams, G. T., McCanahan, T. K., and Morimoto, R. I.: Ela transactivation of the human HSP70 promoter is mediated through the basal transcription complex. *Mol Cell Biol* 9: 2574-2587, 1989
- Wu, B. J. and Morimoto, R. I.: Transcription of the human HSP70 gene is induced by serum stimulation. *Proc Natl Acad Sci USA* 82: 6070-6074, 1985
- Wu, B. J., Hunt, C., and Morimoto, R. I.: Structure and expression of the human gene encoding major heat shock protein HSP70. *Mol Cell Biol* 5: 330-341, 1985
- Wu, B. J., Kingston, R. E., and Morimoto, R. I.: Human HSP70 promoter contains at least two distinct regulatory domains. *Proc Natl Acad Sci USA* 83: 629-633, 1986
- Wurst, W., Benesch, C., Drabent, B., Rothermel, E., Benecke, B.-J., and Günther, E.: Localisation of heat shock protein 70 genes inside the rat major histocompatibility complex close to class III genes. *Immunogenetics* 30: 46-49, 1989
- Yasuhiro, D. and Kishimoto, S.: Elevated expression of heat-shock protein gene in the fibroblasts of patients with scleroderma. *Clin Sci* 78: 419-422, 1990
- Young, R. A.: Stress proteins and immunology. *Annu Rev Immunol* 8: 401-420, 1990